## 1 WHAT IS CLAIMED IS:

- 2 1. A method of producing an assembled peptide in aqueous solution and on a solid phase 3 comprising:
- a) binding an unprotected first peptide segment to a solid phase via a linker, wherein said
- 5 unprotected first peptide segment comprises an N-terminus and a thioester of the formula
- 6 -COSR at its C-terminus, wherein said linker comprises a cleavable moiety and said
- 7 unprotected first peptide segment is bound to said linker at said N-terminus;

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- b) ligating a second unprotected peptide segment to said first peptide segment bound to said solid phase, wherein said second peptide segment comprises a cysteine at its N-
- terminus and a thioacid at its C-terminus, and wherein said N-terminal cysteine of said
- second peptide segment is capable of selectively ligating to said C-terminus of said solid
- phase-bound first peptide segment to form a solid phase-bound peptide comprising a
- thioacid at its C-terminus;

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- c) converting said C-terminal thioacid of said solid phase-bound peptide to an activated
- thioester of the formula -COSR,

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- d) repeating steps b) and c) with a third unprotected peptide segment; and
- e) optionally repeating steps b) and c) with additional unprotected peptide segments.

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- 22 2. The method of claim 1, further comprising, after step e):
- 23 f) cleaving said linker to release an assembled peptide.

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25 3. The method of claim 1, wherein said assembled polypeptide is from 20 to 1000 amino acids 26 in length.

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28 4. The method of claim 1, wherein said solid support is a bead resin.

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30 5. The method of claim 1, wherein said cleavable moiety is a cleavable handle.

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- 2 6. The method of claim 1, wherein said cleavable moiety is a cleavable linker.
- 4 7. The method of claim 1, wherein said peptide segments range in size from 5 to 99 amino acid 5 residues.
- 7 8. The method of claim 1, wherein said peptide segments are all prepared by stepwise solid 8 phase synthesis.
- 10 9. The method of claim 1, wherein the last peptide segment to be ligated onto the solid phase-11 bound peptide is derived from recombinant DNA expression.
- 13 10. The method of claim 1, wherein at least one of said peptide segments comprises an unnatural backbone structure.
- 16 11. The method of claim 1, wherein said converting step is accomplished using bromoacetic 17 acid.
- 19 12. The method of claim 1, further comprising:20 f) monitoring the ligation reactions using mass spectrometric analysis.
- 20 f) monitoring the ligation reactions using mass spectrometric analysis.
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- 22 13. A method of producing an assembled peptide comprising:
- a) binding an unprotected first peptide segment to a solid phase via a linker, wherein said
- 24 unprotected first peptide segment comprises an N-terminus and a thioacid of the formula
- 25 -COSH at its C-terminus, wherein said linker comprises a cleavable moiety and said
- unprotected first peptide segment is bound to said linker at said N-terminus;
- b) converting said C-terminal thioacid of said first peptide segment to a thioester of the
- 28 formula -COSR;
- 29 c) ligating a second unprotected peptide segment to said first peptide segment bound to
- 30 said solid phase, wherein said second peptide segment comprises a cysteine at its N-

1	terminus and a thioacid at its C-terminus, and wherein said N-terminal cysteine of said
2	second peptide segment is capable of selectively ligating to said C-terminus of said solid
3	phase-bound first peptide segment to form a solid phase-bound peptide comprising a
4	thioacid at its C-terminus;
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6	d) converting said C-terminal thioacid of said solid phase-bound peptide to an activated
7	thioester of the formula -COSR,
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9	e) repeating steps c) and d) with a third unprotected peptide segment; and
10	f) optionally repeating steps c) and d) with additional unprotected peptide segments.
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12	14. A method of preparing an assembled peptide comprising:
13	a) binding a first peptide segment to a solid phase via a linker, wherein said first peptide
14	segment comprises an N-terminal Cysteine and a C-terminal residue capable of binding to said
15	linker, wherein said linker comprises a cleavable moiety and said first peptide segment is bound
16	to said linker at said C-terminal residue;
17	b) ligating a second peptide segment to said first peptide segment bound to said solid
18	phase, wherein said second peptide segment comprises a cysteine at its N-terminus and a
	thioester at its C-terminus, and wherein said N-terminal cysteine of said second peptide segment
20	is capable of binding to a protecting group; and wherein said C-terminal thioester of said second
21	peptide segments binds to said N-terminal cysteine of solid phase-bound first peptide segment to
22	form a solid phase-bound peptide comprising protected Cysteine at its N-terminus;
23	c) removing said protecting group;
24	d) repeating steps b) and c) at least once with another peptide segment;
25	e) ligating a final peptide segment to said solid phase-bound peptide, wherein said fina
26	peptide segment is unprotected and comprises a C-terminal thioester;
27	f) cleaving said cleavable moiety to release an assembled peptide from the solid phase.
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30 step using mass spectrometric analysis of the solid phase-bound peptides.

The method of claim 14, further comprising: monitoring said ligation reactions at any

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2 16.	A method of preparing an assembled peptide comprisin	g:
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- a) binding a first peptide segment to a solid phase via a linker, wherein said first peptide
- 4 segment comprises an N-terminal Cysteine and a C-terminal residue capable of binding to said
- 5 linker, wherein said linker comprises a cleavable moiety and said first peptide segment is bound
- 6 to said linker at said C-terminal residue;
- b) ligating a second peptide segment to said first peptide segment bound to said solid
- 8 phase, wherein said second peptide segment comprises a peptide comprising a C-terminal
- 9 thioester, wherein said C-terminal thioester of said second peptide segment binds to said N-
- 10 terminal cysteine of solid phase-bound first peptide segment to form a solid phase-bound peptide
- 11 comprising protected Cysteine at its N-terminus.

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- 13 17. A method of preparing an assembled peptide comprising:
- a) binding a first peptide segment to a solid phase via a linker to form a solid phase-
- 15 bound peptide,
- a) ligating at least a second peptide segment to said solid phase-bound peptide in aqueous
- 17 solution to form a solid phase-bound peptide.

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19 18. The method of claim 17, wherein said solid phase is water-compatible.

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- 21-19. The method of claim 17, wherein said aqueous solution comprises 1-8 M urea.
- 22 20. The method of claim 17, wherein said aqueous solution comprises 1-6 M guanidine• HCl.
- 23 21. The method of claim 17, wherein said aqueous solution comprises 10-60% acetonitrile in
- 24 water.
- 25 22. The method of claim 17, wherein said aqueous solution comprises a mixed
- 26 aqueous/organic solvent.

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28 23. A kit for preparing assembled polypeptides comprising:

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1	a) a first unprotected peptide segment, comprising a thioester at its C-terminus and an N-
2	terminus, wherein said first unprotected peptide segment is bound to a solid phase via a linker
3	comprising a cleavable moiety;
4	b) a set of second unprotected peptide segments, each comprising a thioacid at their C-
5	termini and a cysteine at their N-termini, wherein each of said second unprotected peptide

- 6 segments have the same number of amino acids; and
  7 c) one or more sets of different unprotected peptide segments, each comprising a thioester
  8 at their C-termini and a cysteine residue at their N-termini, wherein each of the members of each
- 9 set have the same number of amino acids.
- 11 24. The kit of claim 12, wherein said set of second unprotected peptide segments is comprised 12 of peptides having the same length, but different amino acid sequences.
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  14 25. The kit of claim 12, wherein said set of second unprotected peptides consists essentially of
  15 identical peptides.
- 17 26. The kit of claim 13, wherein said one or more sets of different unprotected peptides 18 comprise at least one set of peptides having the same length but different amino acid sequences.
- 20 27. An apparatus for producing assembled polypeptides, comprising:
- a) a solid support, having bound thereto a first unprotected peptide having a thioester at its C-terminus and a cleavable linker at its N-terminus, wherein said unprotected peptide is bound to said solid support via a linker;
- b) a set of second unprotected peptides, each comprising a thioacid at their C-termini and a cysteine residue at their N-termini; and
- c) one or more sets of different unprotected peptides, each comprising a thioacid at their C-termini and a cysteine residue at their N-termini.

1	28.	A	method	of producing	g polypeptide	libraries	of	solid	phase	sequentially	ligated	assemble
2	pept	ide	es, comp	rising:								

- 4 a) covalently binding a set of unprotected first peptide segments to a solid support via a linker,
- 5 wherein said linker comprises a cleavable moiety stable under ligating conditions and said
- 6 unprotected first peptide segments are each bound to said linker at its N-terminus and has a
- 7 thioester at its C-terminus;

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- 9 b) introducing a set of second unprotected peptide segments, wherein each of said second
- 10 segments each comprise a cysteine residue at its N-terminus and a thioacid at its C-terminus,
- 11 under conditions suitable to permit ligation between said first unprotected peptide segments and
- 12 said second unprotected peptide segments to form a natively ligated polypeptide bound to said
- 13 solid support, wherein said bound polypeptide comprises a thioacid at its C-terminus;

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15 c) converting said thioacid to a thioester;

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17 d) optionally repeating steps b) and c) with additional sets of unprotected peptide segments.

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